samples also include those samples prepared in the laboratory, such as those used for research purposes. Cells, if present, may be removed by methods such as centrifugation or lysis. One skilled in the art would readily appreciate the variety of test samples that may be examined for capsid protein and anti-capsid protein antibodies. Test samples may be obtained by such methods as withdrawing fluid with a needle or by a swab. One skilled in the art would readily recognize other methods of obtaining test samples.

An "antibody composition" refers to the antibody or antibodies required for the detection of the protein. For example, the antibody composition used for the detection of capsid protein in a test sample comprises a first antibody that binds to capsid protein as well as a second or third detectable antibody that binds the first or second antibody, respectively.

To examine a test sample for the presence of anti-capsid protein antibodies, a standard immunometric assay may be performed. 10-50 μg/ml of capsid protein is added to a solid phase support, such as a 96-well microtiter plate, in a volume of buffer. 50 μl are added per well. The solid phase support is incubated for a period of time sufficient for binding to occur and subsequently washed with phosphate-buffered saline (PBS) to remove unbound capsid protein. Examples of appropriate conditions are 2 hours at room temperature or 4 °C overnight. The solid phase support is then blocked with a PBS/BSA solution to prevent proteins in the test sample from nonspecifically binding the solid phase support. Serial dilutions of test sample are added to the solid phase support which is subsequently incubated for a period of time sufficient for binding to occur. The solid phase support is washed with PBS to remove unbound protein. Labeled anti-human antibodies, which recognize the Fc region of human antibodies, are added to the solid phase support mixture. The plate is incubated for a period of time sufficient for binding to occur and subsequently washed with PBS to remove unbound labeled anti-human antibody. The amount of bound labeled anti-human antibodies is subsequently determined by standard techniques. The anti-human antibodies that may be used include horseradish peroxidase-labeled, goat anti-human antibodies (Boehringer Mannheim), used at 1:12,000 according to the manufacturer's directions.

To examine a test sample for the presence of capsid protein, a standard immunometric assay such as the one described below may be performed. A first anti-capsid protein antibody, which recognizes a specific portion of capsid protein is added to a 96-well microtiter plate in a volume of buffer. The plate is incubated for a period of time sufficient for binding to occur and subsequently washed with PBS to remove unbound anti-capsid protein antibody. The plate is

then blocked with a PBS/BSA solution to prevent sample proteins from nonspecifically binding the microtiter plate. Serial dilutions of test sample are subsequently added to the wells and the plate is incubated for a period of time sufficient for binding to occur. The wells are washed with PBS to remove unbound protein. Labeled anti-capsid protein antibodies, which recognize portions of capsid protein not recognized by the first anti-capsid protein antibody are added to the wells. The plate is incubated for a period of time sufficient for binding to occur and subsequently washed with PBS to remove unbound, labeled anti-capsid protein antibody. The amount of bound labeled anti-capsid protein antibody is subsequently determined by standard techniques. A rabbit anti-capsid antibody that recognizes capsid protein is used at 1:1000. Examples of appropriate conditions are 2 hours at room temperature or 4°C overnight.

Kits which are useful for the detection of capsid protein in a test sample, comprise solid support, positive and negative controls, buffer, appropriate anti-capsid protein antibodies and instructions for carrying out the capture ELISA assay essentially as previously described. Kits which are useful for the detection of anti-capsid protein antibodies in a test sample, comprise solid support, positive and negative controls, buffer, capsid protein and instructions for carrying out the capture ELISA assay essentially as previously described.

While the portions of the disclosure herein which relate to therapeutic compositions and methods primarily relate to therapeutics and methods of treating humans, the compositions and methods of the present invention can be applied to veterinary medical uses as well. It is within the scope of the present invention to provide methods of treating non-human as well as human individuals. Accordingly, the present invention relates to a method of treating all animals, particularly mammalian species including human, bovine, ovine, porcine, equine, canine and feline species.

The invention is further illustrated by way of the following examples which are intended to elucidate the invention. These examples are not intended, nor are they to be construed, as limiting the scope of the invention. It will be clear that the invention may be practiced otherwise than as particularly described herein. Numerous modifications and variations of the present invention are possible in view of the teachings herein and, therefore, are within the scope of the invention.

EXAMPLES

Example 1: Capsid Protein Expression Constructs.

Two WNV capsid protein (Cp) expression vectors (pWNVh-DJY and pWNVy-DJY) were constructed, based on the reported polyprotein gene sequence for the New York 1999 human isolate of the virus (WNV-HNY1999) (GenBank accession number AF202541, Jia *et al.*, 1999, Lancet, 354:1971-1972). The genomic organization of WNV-HNY1999 is presented in the top portion of Figure 1. The construction of the vectors is presented schematically in the bottom portion of Figure 1. Each construct contains the coding sequence for a signal peptide (leader sequence) from a human IgE (sIgE) fused 5' upstream of the Cp open reading frame (ORF), minus the coding sequence for the first amino acid of Cp (the first amino acid residue (met) is deleted). The clones were constructed using an overlapping PCR approach with three separate PCR reactions, using primer sets designed to introduce species-optimized codons (Kim *et al.*, 1997, Gene, 199:293-301, which is incorporated herein by reference) into the final constructs. The pWNVh-DJY construct contains human-optimized codons for the entire fused sIgE signal peptide/Cp coding sequence. The pWNVy-DJY construct contains yeast-optimized codons for the signal peptide and codons for Cp protein amino acid residues 2 through 6, and human-optimized codons for the rest of the Cp coding sequence. In addition, a proper Kozak sequence was introduced upstream of the signal peptide coding sequence, by use of the PCR primers. Each coding sequence was cloned into pcDNA3.1/V5-HisC (Invitrogen, San Diego, CA), between the HindIII and NotI polycloning sites, to yield expression constructs under the control of the CMV promoter that will express a Cp-His tag fusion protein. Both constructs encode identical proteins having an amino-terminal sIgE leader peptide, fused to amino acids 2 through 123 of WNV Cp protein, followed by the V5 epitope, and a polyhistidine carboxy-terminal tail.

The overlapping PCR construction made use of the following ten primers:

Primer 1. sIgh-VChU1+ (90mer)

ATGGACTGGACCTGGATCCTGTTCCTGGTGGCCGCCGCCACCCGCGTGCACAGCT CTAAGAAACCAGGAGGCCCCGGCAAGAGCCGCGCC (SEQ ID NO:14).

Primer 2. sIgy-VCyU1.1+ (90mer)

ATGGATTGGACTTGGATCTTATTTTTAGTTGCTGCTGCTACTAGAGTTCATTCTTC TAAAAAACCAGGTGGCCCCGGCAAGAGCCGCGCC (SEQ ID NO:15).

Primer 3. sIgh-VChL1- (88mer)